

WHAT IS CLAIMED IS:

1. (currently amended) A method for exponentially and selectively amplifying a target nucleic acid in a helicase-dependent reaction, the method comprising:
 - (a) providing single strand templates of the target nucleic acid to be amplified;
 - (b) adding oligonucleotide primers for hybridizing to the templates of step (a);
 - (c) synthesizing an extension product of the oligonucleotide primers which are complementary to the templates, by means of a DNA polymerase to form a duplex;
 - (d) contacting the duplex of step (c) with a helicase preparation for unwinding the duplex such that the helicase preparation comprises a helicase and a single strand binding protein (SSB) unless the helicase preparation comprises a thermostable helicase wherein the single strand binding protein is optional; and
 - (e) repeating steps (b)-(d) to exponentially and selectively amplify the target nucleic acid in a helicase-dependent reaction such that amplification does not occur in the absence of the helicase as determined by gel electrophoresis.
2. (original) A method according to claim 1, wherein amplification is isothermal.
3. (cancel) A method according to claim 1, wherein the target nucleic acid of step (a) is a single stranded nucleic acid.

4. (previously presented) A method according to claim 1, wherein the target nucleic acid is a DNA.

5. (currently amended) A method according to claim 31, wherein the target nucleic acid is an RNA.

6. (original) A method according to claim 1, wherein the target nucleic acid is a double-stranded nucleic acid, the double-stranded nucleic acid having been denatured by heat or enzymatically prior to step(a).

7. (original) A method according to claim 1, wherein the target nucleic acid has a size in the range of about 50bp to 100kb.

8. (original) A method of claim 1, wherein the oligonucleotide primers are a pair of oligonucleotide primers wherein one primer hybridizes to 5'-end and one primer hybridizes to 3'-end of the target nucleic acid to be selectively amplified.

9. (original) A method according to claim 1, wherein the oligonucleotide primers have a length and a GC content so that the melting temperature of the oligonucleotide primers is about 10°C-30°C above the reaction temperature of hybridization during amplification.

10. (original) A method according to claim 9, wherein the DNA polymerase is selected from a Klenow fragment of *E. coli* DNA polymerase I, T7 DNA polymerase (Sequenase) and Bst polymerase large fragment.

11. (original) A method according to claim 10, wherein the DNA polymerase lacks 5' to 3' exonuclease activity.

12. (original) A method according to claim 11, wherein the DNA polymerase possesses strand displacement activity.

13. (original) A method according to claim 1, wherein the helicase preparation comprises a single helicase.

14. (withdrawn) A method according to claim 1, wherein the helicase preparation comprises a plurality of helicases.

15. (withdrawn) A method according to claim 1, wherein the helicase preparation comprises a 3' to 5' helicase.

16. (withdrawn) A method according to claim 1, wherein the helicase preparation comprises a 5' to 3' helicase.

17. (withdrawn) A method according to claim 1, wherein the helicase preparation comprises a superfamily 1 helicase.

18. (withdrawn) A method according to claim 1, wherein the helicase preparation comprises a superfamily 4 helicase.

19. (withdrawn) A method according to claim 1, wherein the helicase preparation is selected from a superfamily 2 helicase, a superfamily 3 helicase, and an AAA⁺ helicase.

20. (withdrawn) A method according to claim 1, wherein the helicase preparation comprises a hexameric helicase.

21. (withdrawn) A method according to claim 1, wherein the helicase preparation comprises a monomeric or dimeric helicase.

22. (withdrawn) A method according to claim 1, wherein the helicase preparation comprises a UvrD helicase or homolog thereof.

23. (withdrawn) A method according to claim 22, wherein the UvrD helicase comprises a thermostable helicase or homolog thereof

24. (withdrawn) A method according to claim 1, wherein the helicase preparation comprises one or more helicases selected from the group consisting of: a UvrD helicase, a recBCD helicase, *E. coli* UvrD helicase, Tte-UvrD helicase, T7 Gp4 helicase, RecBCD helicase, DnaB helicase, MCM helicase, Rep helicase, RecQ helicase, PcrA helicase, SV40 large T antigen helicase, Herpes virus helicase, yeast Sgs1 helicase, DEAH_ ATP-dependent helicases and Papillomavirus helicase E1 protein and homologs thereof.

25. (withdrawn) A method according to claim 22, wherein the UvrD helicase is *E.coli* UvrD helicase.

26. (withdrawn) A method according to claim 23, wherein the thermostable helicase is Tte-UvrD helicase.

27. (withdrawn) A method according to claim 1, wherein the helicase preparation comprises a RecBCD helicase.

28. (withdrawn) A method according to claim 14, wherein the helicase preparation comprises T7 gene 4 helicase and *E. coli* UvrD helicase.

29. (original) A method according to claim 1, wherein the energy source in the helicase preparation is selected from adenosine triphosphate (ATP), deoxythymidine triphosphate (dTTP) or deoxyadenosine triphosphate (dATP).

30. (original) A method of claim 29, wherein the ATP, dATP or dTTP are at a concentration in the range of about 0.1-50mM.

31. (previously presented) A method according to claim 1, wherein the helicase preparation comprises a single strand binding protein.

32. (previously presented) A method according to claim 31, wherein the single stranded binding protein (SSB) is selected from T4 gene 32 SSB, *E.coli* SSB, T7 gene 2.5 SSB, phage phi29 SSB and derivatives therefrom.

33. (original) A method according to claim 1, wherein the helicase preparation comprises an accessory protein.

34. (withdrawn) A method according to claim 33, wherein the accessory protein for a UvrD helicase is MutL.

35. (withdrawn) A method according to claim 1, wherein the helicase preparation comprises *E. coli* UvrD helicase, ATP, *E. coli* MutL protein and T4Gp32.

36. (withdrawn) A method according to claim 1, wherein the helicase preparation comprises *E.coli* RecBCD, ATP, and T4 Gp32 SSB.

37. (withdrawn) A method according to claim 1, wherein the helicase preparation comprises T7 Gp4B helicase, dTTP, and T7 Gp2.5 SSB.

38. (withdrawn) A method according to claim 1, wherein the helicase preparation comprises the thermostable Tte-UvrD helicase, dATP or ATP.

39. (withdrawn) A method according to claim 1, wherein the helicase preparation comprises the thermostable Tte-UvrD helicase, dATP or ATP and T4 gp32 SSB.

40. (original) A method according to claim 1, wherein steps (b) - (e) are performed at a substantially single temperature in the range of about 20°C - 75°C.

41. (original) A method according to claim 1, wherein steps (b)-(e) are performed at about 37°C.

42. (previously presented) A method according to claim 23, wherein steps (b)-(e) are performed at about 60°C and the helicase in the helicase preparation is a thermostable helicase.

43. (original) A method according to claim 1, wherein the target nucleic acid is obtained from a pathogen in a biological sample, and step (e) further comprises amplifying the target nucleic acid to detect the pathogen.

44. (original) A method according to claim 1, wherein the target DNA is chromosomal DNA and step (e) further comprises detecting a sequence variation in the chromosomal DNA.

45. (original) A method according to claim 44, wherein the sequence variation is a single nucleotide polymorphism.

46. (withdrawn) A nucleic acid amplification kit, comprising: a helicase preparation; a DNA polymerase; and instructions for performing helicase dependent amplification according to claim 1.

47. (withdrawn) A nucleic acid amplification kit according to claim 46, wherein the helicase preparation comprises:

a UvrD helicase, a single strand binding protein and adenosine triphosphate, for performing amplification according to claim 1.

48. (withdrawn) An isothermal amplification system that can amplify a target sequence larger than about one thousand nucleotides.

49. (currently amended) A method for determining whether a helicase is suited for exponentially and selectively amplifying a target nucleic acid in a helicase-dependent reaction, comprising;

- (a) preparing a helicase preparation comprising the helicase, an NTP or dNTP, a buffer, wherein the buffer has a pH in the range of about pH 6.0- 9.0, a concentration of NaCl or KCl in a concentration range of 0-200mM, and Tris-acetate or Tris-HCl and ~~optionally one or more single strand binding proteins and~~ an accessory protein to which is added one or more single strand binding proteins unless the helicase is thermostable wherein the single strand binding protein is not required;
- (b) adding a target nucleic acid, oligonucleotide primers, four dNTPs and a DNA polymerase to the helicase preparation.

- (c) incubating the mixture at a temperature between about 20°C and 75°C; and
- (d) analyzing the DNA on an agarose gel to determine whether selective and exponential amplification has occurred.

50. (original) A method according to claim 49, further comprising optimizing the conditions of helicase dependent amplification by varying the concentration of any or each of: the helicase; the single stranded binding protein; the accessory protein; the NTP or dNTP; the salt concentration; the pH; and varying the buffer type; the temperature; the time of incubation and the length of the target nucleic acid.

51. (new) A method for helicase-dependent amplification of a target nucleic acid, comprising:

(a) adding to the target nucleic acid, a helicase preparation comprising a helicase and a single strand binding protein unless the helicase is a thermostable helicase in which case the single strand binding protein is not required, a plurality of oligonucleotide primers and one or more polymerases; and

(b) amplifying, by helicase-dependent amplification, the target nucleic acid wherein the amplification does not occur in the absence of the helicase as determined by gel electrophoresis.